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PHYTOCHEMICAL SCREENING AND MINERAL ELEMENT ANALYSIS OF THE ROOT BARK OF *Parinari macrophylla Sabine* (Chrysobalanaceae) AND ITS EFFECT ON MICROORGANISMS.

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ABSRACT

Parinari macrophylla (Sabine) Chrysobalanaceae, has been use extensively in the Northern part of Nigeria in Ethnomedicine. It is used to treat numerous diseases which include: Asthma, Skin infections, treatment of wounds, pulmonary troubles, dysentery, inflammations and it is also used for the treatment of eye and ear infections. The result of the Phytochemical analysis revealed the presence saponins, tannins, flavonoids, steroids, alkaloids, cardiac glycosides and anthraquinones. The antimicrobial activity of ethanolic extract (30 mg/ml) obtained from *P. macrophylla* showed that the extract had good antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus fumigatus* and *Aspergillus flavus*. The zone of inhibitions produced ranges between 16 mm and 17 mm for the bacterial strains while zone of inhibitions of 30 mm and 35 mm was produced by the fungal strains. The MIC produced, ranges between 0.68 mg/ml and 2.20 mg/ml for the bacterial strains while the MIC of 0.25 mg/ml was produced by the fungal strains. The results of mineral element analysis showed the presence of the following elements at various concentrations: Cu, Cd, Pb, Mn, Mg, Fe, Zn, K, Ca and Na. This present research has validate the ethnomedical uses of the plant in the treatment of skin infections.

KEYWORDS: *Parinari macrophylla*, Phytochemical analysis, Mineral Element Analysis, Antimicrobial Activity and Microorganisms.

INTRODUCTION

As the world tends towards researching into medicinal plants, we have discovered *Parinari macrophylla* (Sabine) Chrysobalanaceae, which has been use extensively in the Northern part of Nigeria in Ethnomedicine. It is used to treat numerous diseases which include: Asthma, Skin infections, treatment of wounds, pulmonary troubles, dysentery, inflammations and it is also used for the treatment of eye and ear infections(Audu et al.,2005). *P. macrophylla* is a tree up to 10m high. The plant is obtained in the coastal strip from Senegal to Liberia and to 300km inland in sandy localities and 700-1000 km inland in mailto Niger and Northern Nigeria. The tree appears to survive annual bush fire in Savanna area and in northern sierra-Leon as the only tree of any stature attaining double the normal 20 m, with a girth of 1.30 m (Keay, 1989). The wood is light brown and firmly hard. *P. macrophylla* have been used by many rural dwellers but have been evaluated scientifically for its ethnomedical usage, phytochemical constituents and its elemental composition. This present study, seeks to provide these information about the the plant.

MATERIALS AND METHODS

COLLECTION OF THE PLANT MATERIALS.

Parinari macrophylla was collected from Birnin Kebbi, Kebbi State, North-Eastern, Nigeria. The plant was identified and authenticated by Auwal Umaru at the herbarium unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The parts of the plant collected were: the leaves, the stem, the root bark, the fruits and the flowers for the purpose of identification.

DRYING AND STORAGE OF THE SAMPLE

The root bark of *Parinari macrophylla* was air dried, and then grounded to powder with aid of pestle and mortar. The root bark was stored in polythene bag until required for use. EXTRACTION OF THE PLANT

MATERIALS

One hundred gram (100 g) of the powdered root bark was extracted with 300 ml of ethanol, using soxhlet extractor. The resulting extract was evaporated to dryness using a rotator evaporator. The percentage yield of the extract was twenty percent (20%).

PHYTOCHEMICAL ANALYSIS

The phytochemical screening was carried out on the ethanol extract of the root bark of *Parinari macrophylla* using the standard methods outlined in Brain and Turner (1975), Trease and Evans (2005) and Harbone(1975).

MINERALIZATION OF SAMPLES.

For the conversion of solid to liquid, a wet digestion technique was used. 0.5 g of the fine powdered samples were placed in beakers for digestion. The contents of the beakers were treated with a mixture of HNO_3 and H_2O_2 in the ratio of 1:1. The beakers with their contents were placed on hot plates in a fume cupboard and heated electrically to boil until all the brown fumes of NO_2 disappeared, leaving behind a colourless liquid. After mineralization, samples were transferred quantitatively to 50 mls volumetric flask and made to mark with de-ionized distil water.

Elemental Analysis.

The aliquots of the digested samples were analysed for metals of interest using Atomic Absorption Spectrophotometer (AAS). Qualitative analysis of the samples were achieved by interpolating the relevant calibration curves prepared from standard metal solution of the aqueous standards.

ANTIMICROBIAL SCREENING

Test Organisms

The test organisms were standard strains of *Aspergillus fumigatus*, *Aspergillus flavus*, *Staphylococcus aureus* and *Escherichia coli*. The organisms were obtained from the Department of Biological Sciences (Mycology Laboratory) and Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Nigeria.

Antimicrobial Activity

Well diffusion method (Oboh *et al*., 2007) was used. The sterilized media was poured into petri dishes. The solidified plates were flooded with the various dilution of the test microorganisms and drained with sterile Pasteur pipette. Wells measuring 8.0mm in diameter were bored into the inoculated plates using cork borer (No.4). The wells were filled with 30 mg/ml of the ethanolic extract. Distilled was used as positive control. Ampicillin was used as negative control for the bacterial strains while fluconazole was used as negative control for the fungal strains. The plates were then incubated at room temperature (27^0C-30^0C) for 18 hfor antibacterial activity. For antifungal activity, the plates were observed on daily basis for any activity for five days. The results were obtained using linear measurement with the aid of a calibrated ruler. The results were measured to the nearest millimeter.

Minimum Inhibitory Concentration (MIC)

Two fold serial dilution of 2 mls of the ethanol extract was made in nutrient broth. Ten dilutions were made and were inoculated with 0.5mls suspensions of the micoorganisms and incubated for 24 h at 37^oC. After incubation, subcultures of the mixtures were made unto nutrient agar plate and incubated for 24 h at 37^oC (i.e for bacterial strains) and for five days for fungal strains.The minimal inhibitory concentration (MIC), is defined as the lowest concentration that produced no visible bacterial or fungal growth after the incubation time was recorded.

Table 1: Phytochemical Constituents of the root bark of *Parinari macrophylla.*

Constituent	Root Bark
Alkaloids	+
Anthraquinones	+
Tannins	+
Steroids	+
Flavonoids	+
Saponins	+
Cardiac glycosides	+

Note: + = Present and - = Absent

TABLE 2: Antimicrobial Activities of the Ethanolic Extract of *Parinari macrophylla* (Zone of inhibition of growth in mm)

Organisms	*P. Macrophylla*	Ampicillin	Fluconazole	Distilled H_2O
Aspergillus fumigatus	35.50	NDa	35.00	-
Aspergillus flavus	35.00	NDa	35.00	-
Staphylococcus aureus	17.00	22.00	NDf	-
Escherichia coli	16.00	17.00	NDf	-

Note : The results are mean of 4 readings
NDa= Not determined since ampicillin has no activity on fungal strains.
NDf=Not determined since fluconazole has no activity on bacterial strains.

Table 3: Minimum Inhibitory Concentration (MIC) of *P. macrophylla* in mg/ml.

Microorganisms	MIC
Staphylococcus aureus	0.68
Escherichia coli	2.20
Aspergillus flavus	0.25
Aspergillus fumigates	0.25

Table 4: The concentrations of the elemental constituents of the root bark of *Parinari macrophylla* expressed in µg/g.

Mineral Element	Root Bark
Cu	52.69
Cd	-0.61
Pb	6.59
Mn	25.15
Mg	335.69
Fe	278.3
Zn	10.74
K	1705.7
Ca	1344.72
Na	-59.00

RESULTS

The results of the Phytochemical analysis revealed the presence of saponins, tannins, flavonoids, steroids, alkaloids, cardiac glycosides and anthraquinones (Table.1). The antimicrobial activity of ethanolic extract (30mg/ml) obtained from *P. macrophylla* revealed that the extract showed a good antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus fumigatus* and *Aspergillus flavus* (Table. 2). The zone of inhibitions produced ranges between 16 mm and 17 mm for the bacterial strains while zone of

inhibition ranges between 30 mm and 35 mm was produced by the fungal strains. (Table. 2). The MIC produced ranges between 0.68 mg/ml and 2.20 mg/ml for the bacterial strains while the MIC of 0.25 mg/ml was produced by the fungal strains (table.3). The results of mineral element analysis showed the presence of the following elements at various concentration with Ca and K having the highest concentrations, then followed by Mg and Fe. Cd has the lowest concentration (Table.4).

DISCUSSION

In the present study, *P. macrophylla* was investigated for its antimicobial activity against *Staphylococcus aureus, Escherichia coli, Aspergillus fumigatus* and *Aspergillus flavus*, *P. macrophylla* is a medicinal plant commonly used in traditional medicine in Northern Nigeria to treat Asthma, Skin infections, treatment of wounds, pulmonary troubles, dysentery, inflammations and it is also used for the treatment of eye and ear infections. The results of the antimicrobial activity showed that the ethanolic extract had some antimicrobial activity both on the bacterial strains and the fungal strains (Table.2). The antimicrobial activity demonstrated by the ethanolic extract may be due to the presence of the secondary metabolites detected in the plant. Phenolic compounds like tannins, flavonoids and anthraquinones have been widely reported to show antimicrobial actions (Sharada *et al*., 2008). According to Audu (2005), the hexane and ethylacetate fractions of *P. macrophylla* showed antibacterial activity. The antifungal activity is reported and is shown on (Table .2). The antifungal activity may be due to the presence of saponins, tannins and flavonoid (Tra Bi *et al*., 2008), (Oboh *et al*., 2008) As it can be seen from table 2, the extract can be compared favorably with fluconazole. The mineral element composition of *P. macrophylla* have been reported for the first time. The evaluation was carried out to determine where the plant has accumulated some toxic, element since the plant is used in medications. As it can be seen from table 4, the levels of the toxic elements (Pb and Cd) are very low when compared with the recommended daily intake of 10mg/kg.

CONCLUSION

Our results offer a scientific basis for the use of *P. macrophylla* in traditional medicine
in Northern Nigeria for treatment of skin disorders. Also the presence some secondary metabolites which may be responsible for the pharmacological activity have been reported. We have also established that the plant has not accumulated toxic elements.

REFERENCES

Audu, O.T., Oyewale, O and Amupitan J.O. (2005). The Biological Activities of Secondary Metabolites of Parinari macrophylla-Sabine. *Chemclass Journal*. 2005, Vol. 2 Pp, 19-21.

Brain, K.R and Turner T.D. (1975). The practical Evaluation of Phytopharmaceuticals. Wright-scientechnica, Pp 90-121.

Evans, W.C.(2005). Trease and Evans Pharmacognosy. 15th Edition. Elsevier India. Pp135-150.

Harbone, J.B. (1973). Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd. London. Pp49-188.

Keay R.W.J. (1989). Trees of Nigeria. Clarendon Press Oxford, London P 181.

Oboh, I.E., Akerele, J.O and Obasuyi, O.(2007). Antimicrobial Activity of The Ethanol Extract of The Aerial Parts of *Sida acuta* burm.f. (malvaceae). *Tropical Journal of Pharmaceutical Research*, 6 (4): 809-813

Sharada L Deore., Khadabadi S.S., Lalita Bhagure and Ghorpada. (2008). In vitro Antimicrobial and Antioxidant Studies on *Enicostemma axillare* (Lam) Raynal leaves. *Natural Product Radiance*, Vol. 7(5), pp, 409- 412.

TraBi, F.H., Koné M.W and Kouamé, N.F. (2008). Antifungal Activity of *Erigeron floribundus* (Asteraceae) From Côte d'Ivoire, West Africa. *Tropical Journal of Pharmaceutical Research*, June 2008; 7 (2): 975-979.

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